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Review

Chitinases of filamentous fungi: a large group of diverse proteins with multiple physiological functions

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ABSTRACT

Chitin is the second most abundant natural biopolymer and the main structural component of invertebrate exoskeletons and cell walls of filamentous fungi. Fungal chitinases have multiple physiological functions including the degradation of exogenous chitin and cell wall remodelling during hyphal growth, but the regulation of the chitinolytic systems of filamentous fungi is not well understood. Fungi have on average between 10 and 25 different chitinases, but only the increasing number of fungal genome sequencing projects in the last few years has enabled us to assess the whole range and diversity of fungal chitinases. In this review the variety, domain architecture and subgroups of chitinases of filamentous fungi are shown, and how these data integrate with that from molecular biological studies on chitinases are discussed.

1. Introduction

Chitin is a linear, insoluble homopolymer composed of β-1,4-linked subunits of the acetylated amino sugar *N*-acetylglucosamine. In nature two major types of chitin occur, which are characterized by an antiparallel (α-chitin) or a parallel (β-chitin) arrangement of the *N*-acetylglucosamine chains (Fig 1). After cellulose, chitin is the second most abundant polymer found in the biosphere (Tharanathan & Kittur 2003). It is the main compound of invertebrate exoskeletons and an essential structural component of the cell walls of filamentous fungi.

Previous reviews of fungal chitinases already emphasized the diversity of these enzymes as well as their multiple functions in fungi (Gooday 1990; Jollès & Muzzarelli 1999). However, only the recent advent of fungal genome sequencing projects has enabled us to assess the whole range and variety of fungal chitinases (http://genome.jgi-psf.org/mic_home.html, http://www.broad.mit.edu/seq/msc/). While in bacterial genome databases on average only between two and four chitinases can be found, the genomes of filamentous fungi typically contain between 10 and 25 different chitinases. The reasons why fungi have so many chitinases are not well understood. Potential physiological roles of fungal chitinases, as already discussed in previous reviews (Adams 2004; Cohen-Kupiec & Chet 1998; Duo-Chuan 2006; Gooday 1990; Jollès & Muzzarelli 1999; Yang *et al.* 2007), include: (i) degradation of exogenous chitin present in fungal cell walls of dead hyphal fragments or in the exoskeletons of dead arthropods, and the use of the degradation products as a nutrient source; (ii) cell wall remodelling during the fungal life cycle, which includes putative roles of chitinases during hyphal growth, branching, hyphal fusion and autolysis; and (iii) competition and defence against other fungi or arthropods in the fungal habitat. Some fungi have even developed lifestyles which

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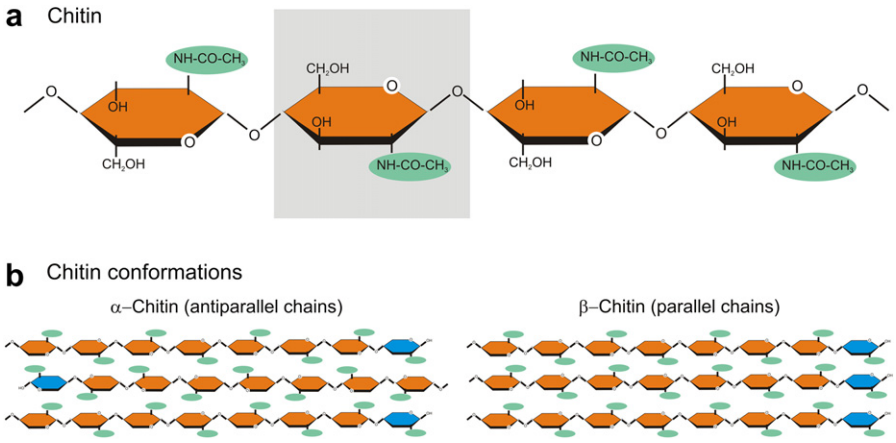

Fig. 1 – (a) Chemical structure of chitin. The grey box indicates one *N*-acetylglucosamine subunit of the chitin chain. (b) The two major types of chitin are characterized by an antiparallel (α-chitin) or parallel (β-chitin) arrangement of the chains.

involve the use of chitinases to actively attack of other fungi (mycoparasitism), insects (entomopathogenic fungi) or nematodes (nematode-trapping fungi).

This review aims at highlighting the lessons that we have learned about chitinases in the past few years from the increasing number of available fungal genome sequences and how these data integrate with the already available knowledge derived from molecular biological studies on chitinases.

2. Mechanisms of chitin degrading enzymes

Chitinolytic enzymes can be divided into *N*-acetylglucosaminidases and chitinases, which substantially differ in their cleavage patterns (Fig 2). *N*-acetylglucosaminidases (EC 3.2.1.52) catalyze the release of terminal, non-reducing *N*-acetylglucosamine (GlcNAc) residues from chitin, but in general they have the highest affinity for the dimer *N*,*N*′-diacetylchitobiose $(GlcNAc)_2$ and convert it into two monomers (Horsch *et al.* 1997). According to the CAZy classification (Carbohydrate Active Enzymes database, Coutinho & Henrissat 1999; http://www.cazy.org) *N*-acetylglucosaminidases belong to glycoside hydrolase (GH) family 20. It is important that those enzymes must not be referred to as exochitinases, which is unfortunately often confused in the literature. In contrast to that, chitinases (EC 3.2.1.14) are members of GH families 18 and 19 and catalyze the hydrolysis of the β-1, 4 linkages in chitin and chitooligomers, resulting in the release of short-chain chitooligomers. GH families 18 and 19 do not share sequence similarity, have different three-dimensional structures (Kezuka *et al.* 2006; Perrakis *et al.* 1994; Terwisscha van Scheltinga *et al.* 1996; van Aalten *et al.* 2000) and different catalytic mechanisms with ß-anomeric products being formed by GH 18 chitinases (retaining mechanism, Brameld *et al.* 1998), whereas α-anomers are produced by GH 19 chitinases (inverting mechanism, Brameld & Goddard 1998). Furthermore, depending on their cleavage patterns, chitinases can be divided into endo- and exochitinases (Fig 2). Endochitinases degrade chitin from any point along the polymer chain forming random-size length products while exochitinases cleave from the non-reducing chain end and the released product is $(GlcNAc)_2$. However, the enzymatic properties of chitinases are more complex and versatile than reflected in the exo-/endo classification. Detailed studies of the chitinolytic system of the bacterium *Serratia marcescens* demonstrated another way to classify the enzymatic properties of chitinases by grouping them into processive and non-processive enzymes (e.g. Horn *et al.* 2006; Sorbotten *et al.* 2005; Uchiyama *et al.* 2001). Processive chitinases do not release the substrate after hydrolytic cleavage but slide it through the active site-tunnel for the next cleavage step to occur. The presence of a carbohydrate binding domain can enhance processivity, but is not essential for it. Non-processive chitinases dissociate completely from the substrate after hydrolysis. This leads for

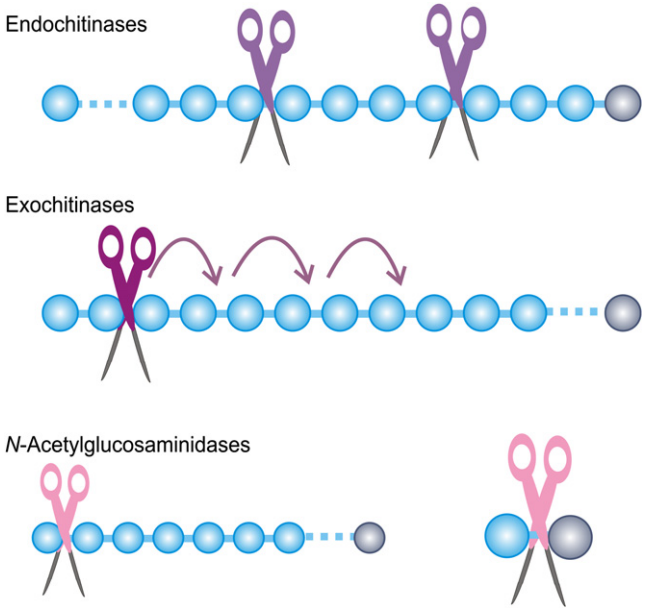

Fig. 2 – Schematic drawing of the predominant cleavage patterns of chitinolytic enzymes. The subunits of the chitin chain are shown in light blue and the reducing end sugar in dark blue. Dotted lines indicate that the polymer substrates are longer than shown in the figure.

non-processive enzymes to substrate degradation patterns with a homogenous distribution of medium chain (6–30mer) products and for processive enzymes to remnants of the polymeric substrate and only 2–8mer short chain degradation products (Horn *et al.* 2006).

Chitinases can contain various carbohydrate-binding modules (CBMs). These CBMs include different families classically defined as chitin- and cellulose-binding domains due to their preferred affinity for these carbohydrates (Henrissat 1999). Extensive data, classification and applications of these CBMs can be found in recent reviews (Boraston *et al.* 2004; Shoseyov *et al.* 2006) and in the Carbohydrate-Binding Module Family Server (http://www.cazy.org/fam/acc_CBM.html).

3. The chitinolytic enzyme machinery of fungi

Completing the picture by in silico *analysis of genomic data*

Analysis of more than 25 fungal genomes so far has shown that fungal chitinases exclusively belong to GH family 18. GH 18 enzymes are not only present in fungi, but also in bacteria, animals, viruses and plants. They were traditionally subdivided into classes III and V, which – because of their predominant occurrence in selected organisms – were also termed fungal/plant (class III) and fungal/bacterial (class V) chitinases. Class III and V chitinases differ in the architecture of their substrate binding pockets (Terwisscha van Scheltinga *et al.* 1994; van Aalten *et al.* 2001). Class V (bacterial-type) enzymes have deep, tunnel-shaped substrate binding grooves, and class III (plant-type) enzymes have shallow, open substrate binding grooves. Consequently class V enzymes show exo-acting activities (corresponding to processive enzymes), whereas class III enzymes are endo-chitinases (non-processive enzymes) (Hoell *et al.* 2005; Hurtado-Guerrero & van Aalten 2007; Jaques *et al.* 2003).

The first generation of a complete list of chitinolytic enzymes based on genomic sequence data was carried out in *H. jecorina* (*T. reesei*) (Seidl *et al.* 2005). It revealed the presence of 18 open reading frames (ORFs) encoding putative chitinases and two additional ORFs encoding low-similarity GH 18 proteins, demonstrating that previous research approaches had missed more than two-thirds of the *Hypocrea/Trichoderma* chitinases. In contrast to that, the ORFs encoding the two orthologues of the already described *N*-acetylglucosaminidases, and only one additional hypothetical protein distantly related to β-*N*-acetylhexosaminidases, were the only GH 20 proteins that could be detected in the *H. jecorina* genome database. These findings reflect a similar situation in other fungi with annotated genomes such as *Neurospora crassa*, *Magnaporthe grisea*, *Aspergillus spp.*, *Fusarium spp.*, *Botrytis cinerea*, *Sclerotinia sclerotiorum*, etc., which have ORFs for a large number of chitinases (ca. 10–25, GH 18) but only between two and three ORFs encoding β-*N*-acetylhexosaminidases (GH 20). This suggests that chitinases rather than *N*-acetylglucosaminidases dictate the chitin depolymerization potential of a fungus and are the key elements for the regulation of different aspects of chitin degradation. However, the total number of chitinases in different fungi is highly variable, ranging from, for example, two chitinases in *Ustilago maydis* to 27 in *Fusarium oxysporum*. Nevertheless, in the majority of ascomycetes and basidiomycetes on average of 15 different chitinase-encoding genes can be detected per species.

Classification into subgroups

Screening of fungal genomes for the presence of ORFs encoding putative chitinases revealed an even larger diversity of these proteins than previously anticipated. A phylogenetic analysis of chitinases from sequenced fungal genomes showed that they can be divided into three different subgroups (Fig 3, Seidl *et al.* 2005). Subgroups A and B (corresponding to classes V and III, respectively) contained all of the previously identified fungal chitinases, whereas subgroup C comprised a novel group of, high molecular weight chitinases that had not yet been described in filamentous fungi.

Subgroup A chitinases contain a catalytic domain, but no CBMs, and have on average a molecular mass of 40–50 kDa. Chitinases from subgroup A are present in all fungal genomes. On average ascomycetes have ca. six and basidiomycetes ca. four subgroup A chitinases, but depending on the total number of chitinases of a fungus, numbers of subgroup A chitinases range from two in *U. maydis* to ca. 12 in *F. oxysporum*. The most strongly conserved and abundantly expressed chitinases of the fungal kingdom can be found in the subgroup A-clade containing *H. jecorina* CHI18-5 (ECH42), and most so far studied chitinases belong to this clade (see also Section 4). Many subgroup A chitinases have an N-terminal signal peptide that targets them to the secretory pathway and are thus extracellular proteins but some are predicted to be intracellular proteins or have an ER-targeting sequence (e.g. *A. nidulans* EAA66094, *N. crassa* XP_959610, *H. jecorina* DAA05852, *G. zeae* EAA76014).

Subgroup B chitinases are very variable in their size and domain structure and their molecular masses range from 30–90 kDa. They can be grouped in small subgroup B chitinases (30–45 kDa) that contain frequently CBMs – a feature that is completely absent from subgroup A chitinases – and in large proteins of ca. 90 kDa that have long, unstructured, serine/threonine rich domains and/or a GPI-anchoring signal indicating that the mature proteins are bound to the plasma membrane. CHIA of *A. nidulans* is an example for such a

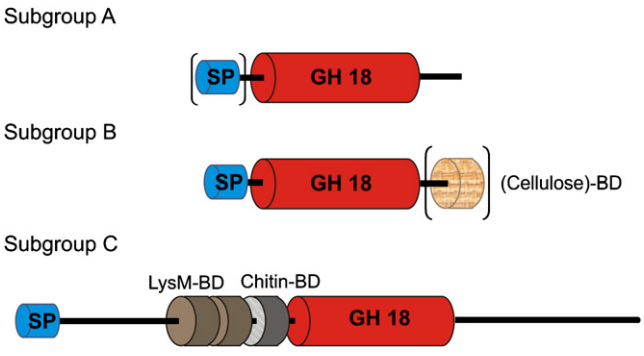

Fig. 3 – Domain organization of fungal chitinases. SP, signal peptide. GH 18, glycoside hydrolase family 18, BD, binding domain.

GPI-anchored chitinase and it has been reported to be involved in cell wall remodelling processes (Takaya *et al.* 1998a).

The number of subgroup B chitinases is strongly variable among different fungal species. In most fungal genomes of ascomycetes (e.g. *N. crassa*, *Mg. grisea*, *A. nidulans*, *A. niger*, *Mycosphaerella fijiensis*, *Myc. graminicola* and *Coccidoides immitis*). and basidiomycetes (*Laccaria bicolor*, *Phanerochaete chrysosporium*, *Postia placenta*) only 2–3 ORFs encoding subgroup B chitinases are present. *Fusarium* spp. have even only one ORF encoding a subgroup B chitinase and no subgroup B chitinases at all seem to be present in the genomes of the basidiomycetes *Coprinus cinereus* and *Sporobolomyces roseus*. In contrast to that, a number of fungi such as *Hypocrea/Trichoderma* spp. (*H. jecorina*, *H. atroviridis*, *H. virens*), *Aspergillus* spp. (*A. fumigatus*, *A. clavatus*, *Neosatorya fischeri*) and *Rhizopus oryzae* have up to five subgroup B chitinases. This indicates that these additional chitinases could have specialized functions in the respective fungi. Some of these proteins even belong phylogenetically to a separate clade that includes in addition orthologues described in the entomopathogenic fungus *Metharizium anisopliae*, which suggests a specialized function in chitin degradation.

Analysis of fungal genome sequences confirmed that several of the small (30–45 kDa) subgroup B chitinases possess CBMs, but interestingly these domains vary depending on the fungal species. The ascomycetes *B. cinerea*, *Hyopocrea/Trichoderma* spp. (*H. jecorina*, *H. virens* and *H. atroviridis*) and *S. sclerotiorum* have several subgroup B chitinases with a CBM 1 domain. So far only two subgroup B chitinases containing CBMs were studied in more detail and both have a CBM 1 domain as well (Baratto *et al.* 2006; Kim *et al.* 2002). These types of CBMs were traditionally described as cellulose-binding domains (CellBDs) and are exclusively found in fungi. However, they seem to have a broader polysaccharide binding spectrum and have also already been reported to bind to chitin (Boraston *et al.* 2004). Limón *et al.* (2004) added the CellBD (CBM 1) from cellobiohydrolase II of *H. jecorina* (*T. reesei*) to the *H. lixii* (*T. harzianum*) chitinases CHI18-12 (CHIT33) and CHI18-5 (CHIT42), and obtained enzymes with stronger chitin-binding capacity. In other fungi, e.g. the zygomycete *Phycomyces blakesleeanus* and the basidiomycetes *L. bicolor*, *Po. placenta* and *Ph. Chrysosporium*, the CBMs of subgroup B chitinases belong to family 5/12, which were originally also classified as CellBDs. Interestingly in *Aspergillus spp.* only few subgroup B chitinases have CBMs. *A. fumigatus*, *A. clavatus* and *A. oryzae* have each one subgroup B chitinase with a CBM 19 (described as having chitin-binding function) but no CBMs can be found in subgroup B chitinases of *A. niger*, *A. nidulans*, *A. terreus*, *A. flavus* and *N. fischeri*.

Subgroup C is a novel subgroup of fungal chitinases. The first chitinase described from this subgroup was *H. atroviridis* (*T. atroviride*) CHI18-10 (Seidl *et al.* 2005). Subgroup C chitinases have typically a molecular mass of 140–170 kDa and despite their large size, which was confirmed by cDNA sequencing for e.g. *H. atroviridis chi18-10* (Seidl *et al.* 2005), they have an N-terminal signal peptide targeting them to the secretory pathway. In addition, subgroup C chitinases contain several features which clearly distinguish them from other fungal chitinases. They have a chitin-binding domain (CBM 18) and multiple LysM-motifs, which are short peptide domains implicated in binding peptidoglycan and structurally related molecules such as chitin in bacterial and eukaryotic proteins, respectively (Ohnuma *et al.* 2007; Zhang *et al.* 2007). The LysM domains of the plant chitinase PrChi-A were shown to be essential for the antifungal activities of the protein (Onaga & Taira 2008).

The number of subgroup C chitinases is strongly variable among different fungal species. While a number of ascomycetes have many subgroup C chitinases, e.g. *F. graminearum* (ca. 7), *A. nidulans* (ca. 9), *Nectria haematococca* (ca. 10) and *H. virens* (ca. 12), other ascomycetes have only a few of these chitinases, e.g. *N. crassa* (3), *Mg. grisea* (2), *My. graminicola* (1) and *My. fijiensis* (1). Members of subgroup C do not seem to be present in basidiomycetes.

Although subgroup C is phylogenetically clearly different from subgroups A and B, low amino acid sequence similarities to subgroup A point towards a processive catalytic mechanism. Nevertheless, the completely different domain architecture of subgroup C chitinases in comparison to subgroups A and B suggests a distinctive role and specialization of these enzymes in chitin degradation events.

In addition to those ORFs in fungal genomes that can be safely predicted to encode GH family 18 proteins with chitinolytic functions, a few ORFs encode GH 18-similar proteins. The genome of *N. crassa* encodes three of these proteins with interesting subcellular localization predictions (XP_964254 (GPI-anchored), XP_960622 (intracellular), and XP_963233) and such GH 18-similar proteins can also be found in other fungal genomes. The amino-acid similarities to the residual fungal GH family 18 chitinases are too low to assign them to subgroups A–C and their functions and chitinolytic abilities have not been studied yet.

4. Regulation and functions of fungal chitinases

Degradation of exogenous chitin

Although chitinolytic enzyme activities have been reported from several fungi (see review by Duo-Chuan 2006), molecular biological studies on the regulation and function of individual filamentous fungal chitinases are generally rather scarce. The published data can be divided into two main aspects: induction by exogenous components and regulation by developmental stimuli.

On the regulation and function of individual, different chitinases during exogenous chitin degradation a substantial number of studies has been carried out in *Hypocrea/Trichoderma*. Some species of this genus are mycoparasites, i.e. they invade and destroy fungal cells and then feed on the dead cell contents, and chitinases have been implicated in cell-wall hydrolysis during mycoparasitic attack (Benitez *et al.* 2004; Hjeljord & Tronsmo 1998). Gene expression studies of chitinases and *N*-acetylglucosaminidases in *H. atroviridis* (*T. atroviride*) revealed that these two types of enzymes are induced and regulated by completely different inducers. Although chitin is poorly utilized by *H. atroviridis*, it induces both, chitinases and *N*-acetylglucosaminidases (Mach *et al.* 1999). The monomer of chitin, *N*-acetylglucosamine, on the other hand, is an excellent carbon source for *H. atroviridis*, but while it does not induce chitinases, it induces *N*-acetylglucosaminidases strongly even at

concentrations as low as 1 mM (Mach *et al.* 1999; Peterbauer *et al.* 2002).

CHI18-5 (ECH42) is among the most strongly conserved chitinases in the fungal kingdom. In *Hypocrea/Trichoderma* spp. it is the most abundant chitinase in culture extracts grown on chitinase inducing carbon sources and *chi18-5* expression is triggered by chitin degradation products, irrespective of whether they originate from exogenous chitin or cell wall autolysis (Mach *et al.* 1999; Seidl *et al.* 2005). In addition *H. atroviridis chi18-5* is induced during stages of the mycoparasitic attack (Carsolio *et al.* 1994; Zeilinger *et al.* 1999). Kullnig *et al.* (2000) determined that the induction of *chi18-5* by cell walls of *R. solani* requires the diffusion of a factor with a size between 12–90 kDa and concluded from their experiments that an as yet unidentified chitinase releases oligomers from the host cell-walls which then act as inducers of further chitinase (such as CHI18-5) formation. On the other hand, *chi18-15* (*chit36*), which is also expressed during similar growth conditions was shown to be induced by a soluble molecule <12 kDa, directly released from *R. solani* cell walls (Viterbo *et al.* 2002). The induction of *chi18-5*, *chi18-12* (*chi33*) and *chi18-15* is not only influenced by the presence of chitin or fungal cell walls, but also regulated by carbon catabolite repression, the nitrogen source and starvation (see also Cell wall remodelling; Carsolio *et al.* 1994; de las Mercedes Dana *et al.* 2001; Mach *et al.* 1999; Seidl *et al.* 2005).

Despite the high expression levels of CHI18-5 during mycoparasitic growth conditions, *chi18-5* gene-knockout studies did not show any alterations in the ability of *H. atroviridis* to overgrow other fungi in mycoparasitism plate confrontation assays (Carsolio *et al.* 1999; Woo *et al.* 1999), although culture filtrates of the knockout-strains had reduced antifungal activities against *B. cinerea in vitro* (Woo *et al.* 1999). This suggests that CHI18-5 is expressed as a consequence of the chitin degradation events during mycoparasitic attack, but that it is not a major determinant for this process. In contrast to the broad inducibility of *chi18-5*, the *H. atroviridis* chitinase-encoding genes *chi18-10* and *chi18-13 (ech30)*, are not induced by colloidal chitin or starvation, but by the presence of complex chitinous carbon sources such as *R. solani* cell walls (Klemsdal *et al.* 2006; Seidl *et al.* 2005), demonstrating a more specific regulation of these *H. atroviridis* chitinases.

In a number of other fungi similar findings have also been reported with respect to the induction of individual chitinases by chitin, antagonization of fungi or insects, and by autolysis were reported. These studies include subgroup A chitinases that all belong phylogenetically to the CHI18-5 clade: *B. cinerea BcchiA* (Choquer *et al.* 2007), *Paecilomyces javanicus PjChi-1* (Chen *et al.* 2007), *Stachybotrys elegans sechi44* (Morissette *et al.* 2003), *Paracoccidioides brasiliensis Pbcts1* (Bonfim *et al.* 2006), *Clonostachys rosea Crchi1* (Gan *et al.* 2007b), *Lecanicillium psalliotae Lpchi1* (Gan *et al.* 2007a) and two subgroup B chitinases from *Metarhizium anisopliae*: *chi2* (Baratto *et al.* 2006) and *chi3* (da Silva *et al.* 2005).

How fungi respond to different chitin-conformations and calcification grades of chitin, and how these differences influence chitinase regulation has not been studied yet. Pure chitin is in general not a good carbon source for fungi and in many studies researchers use colloidal chitin which is more accessible to the fungus because it has undergone acidic hydrolysis pretreatment. Another question that has not been addressed is how fungi that secrete high levels of chitinolytic enzymes protect their own cell walls. The avirulence protein AVR4 from the plant pathogenic fungus *Cladosporium fulvum* has been shown to bind to the chitin of fungal cell walls and protect them against plant chitinases (van den Burg *et al.* 2006). Although no further AVR4 orthologues can be detected in fungal genome databases, it is possible that other fungal cell wall proteins such as hydrophobins fulfil similar functions (Wessels 1997). The cell wall protein QID74 from *T. harzianum* has recently been reported that to be involved in resistance to lytic enzymes (Rosado *et al.* 2007).

Cell wall remodelling

Cell wall remodelling during the fungal life cycle is thought to be regulated by a delicate balance between controlled lysis and synthesis of chitin (Bartnicki-Garcia 2006; Gooday 1990; Merz *et al.* 1999). One would be expect that chitinases that are involved in cell wall remodelling, are not induced by exogenous chitin but are developmentally regulated (e.g. during vegetative growth, by the onset of sporulation) and/or show basal expression levels under all growth conditions. These kinds of transcript patterns have indeed been obtained for several chitinases, e.g. from *H. atroviridis* (Seidl *et al.* 2005, *chi18-2*, *chi18-3*, *chi18-4*; all subgroup A), *B. cinerea* (Choquer *et al.* 2007, *BcchiB*; subgroup A), *Rhizopus oligosporus* (Takaya *et al.* 1998b, *chi3*, subgroup A). However, morphological changes in knockout-strains have only been reported for one chitinase encoding gene, *A. nidulans chiA* (subgroup B). The respective strains displayed a decreased hyphal growth rate and lower germination frequency, whereas hyphal and conidiophore morphology were normal (Takaya *et al.* 1998a).

Transcriptional evidence for the induction of chitinases by starvation and subsequent autolysis has been obtained in *H. atroviridis* (*chi18-5*, subgroup A; Mach *et al.* 1999; Seidl *et al.* 2005), *chi18-12* (*chit33*, subgroup B; de las Mercedes Dana *et al.* 2001; Limón *et al.* 1995), in *Aspergillus nidulans* (*chiB*, subgroup A; Pusztahelyi *et al.* 2006; Yamazaki *et al.* 2007) and in *A. fumigatus* (*chiB1*, subgroup A; Jaques *et al.* 2003). Again, only in one case, *A. fumigatus chiB1*, the autolytic function of a chitinase was demonstrated with gene-knockout strains, which showed much lower levels of chitinase activity during the autolytic phase of batch cultures (Jaques *et al.* 2003).

It has been repeatedly suggested (Adams 2004; Hurtado-Guerrero & van Aalten 2007; Jaques *et al.* 2003) that subgroup B chitinases could rather be the ones responsible for cell wall remodelling in fungi. This suggestion is based on the finding that knockout strains of *A. nidulans chiA*, which encodes a subgroup B chitinase, but not of *A. fumigatus chiB*, which encodes a subgroup A chitinase, displayed morphological defects. However, as detailed above (Section 3), subgroup B consists of two types of proteins. While some chitinases, such as *A. nidulans chiA* are large proteins with unstructured serine/threonine rich domains and/or GPI anchors, the majority of subgroup B chitinases are small proteins that can contain a CBM. At least some subgroup B chitinase-encoding genes such as *H. atroviridis chi18-13* have a carbon source-inducible regulation, whereas, a number of subgroup A chitinase-encoding genes such as *chi18-2*, *chi18-3*, *chi18-4* and *chi18-5*, showed transcript levels under all tested growth conditions, which is rather

a transcription pattern that would be expected from chitinases involved in cell wall remodelling. In view of these data, it seems likely that not a certain subgroup of chitinases, but rather selected chitinases from all subgroups have functions in hyphal growth-related processes.

5. Conclusions

Filamentous fungi contain a large number of chitinases and genomic data mining has enabled us to form a detailed picture of the chitinolytic enzyme machinery of fungi, and has revealed an even higher variety of chitinases than previously anticipated. Fungal chitinases belong to GH family 18 and based on a phylogenetic analysis they can be divided into three subgroups (A, B and C), which have distinctive domain architectures (Fig 3). The potential roles of fungal chitinases are exogenous chitin degradation, defence and attack mechanisms against other fungi and arthropods and cell wall remodelling. Major questions to be addressed in the future are: (1) do these enzymes have specialized roles in one or more of the biological processes that chitinases have been implicated as being involved in, (2) do their functions partially overlap, (3) how do fungi regulate their large battery of chitinases, and (4) which signals trigger their expression. The rapid advances in-omics techniques and in different interdisciplinary approaches will hopefully enable us in the next few years to further extend our knowledge about this group of enzymes and their versatile functions.

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